

Amendments to the Specification

Please replace page 40 of the specification as follows:

Cells Lines (No/L)													
Primary Screening		Dose 10		Dose 20		Dose 30		Dose 40		Dose 50		Dose 60	
		10 ⁴	10 ⁵	10 ⁶	10 ⁷	10 ⁸	10 ⁹	10 ¹⁰	10 ¹¹	10 ¹²	10 ¹³	10 ¹⁴	10 ¹⁵
PH-	60	4.9E-05	1.0E-07		8.8E-07	2.0E-05	8.2E-07	5.8E-07		4.7E-05	1.0E-07	4.7E-08	1.0E-05
TS	73	8.6E-05	6.0E-07		2.3E-05	7.0E-05	2.6E-05	9.5E-07		8.8E-05	4.6E-07	1.0E-05	2.7E-05
ZK	128	1.2E-05	1.2E-05		1.2E-05	1.3E-05	8.8E-05	2.0E-05		1.6E-05	1.8E-05	> 1.9E-05	1.8E-05

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

HRFABMS m/z 509.275351 [M-H₂O+H]⁺ (calcd for C₂₈H₃₇N₄O₅ 509.276396 Δ 1.0 mmu); LRFABMS using m-NBA as matrix m/z (rel intensity) 509 [M-H₂O+H]⁺ (5), 460 (2.7), 391 (3).

¹H NMR (CD₃OD, 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, J = 2.0 Hz, H-21), 4.62 (dd, J = 8.4, 3.7 Hz, H-1), 3.98 (bd, J = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, J = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, J = 7.4 Hz, H-26);

¹³C NMR (CD₃OD, 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C-17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-22B	GI50	4.58E-06	3.08E-07	8.49E-07	3.02E-06	8.24E-07	5.20E-07	4.71E-06	1.13E-07	4.77E-06	1.01E-06	2.54E-06	6.95E-06	7.61E-07	4.65E-07
	TGI	8.62E-06	6.08E-07	2.30E-06	7.04E-06	2.28E-06	9.99E-07	8.83E-06	4.67E-07	1.17E-05	2.75E-06	6.84E-06	1.90E-05	1.83E-06	9.32E-07
	LC50	1.62E-05	1.20E-06	1.21E-05	1.65E-05	8.85E-06	2.01E-06	1.66E-05	1.84E-06	>1.90E-05	1.86E-05	1.84E-05	>1.90E-05	7.42E-06	1.86E-06

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): 10 mm inhibition zone

Spectroscopic data:

HRFABMS m/z 509.275351 $[M-H_2O+H]^+$ (calcd for $C_{28}H_{37}N_4O_5$ 509.276396 Δ 1.0 mmu);

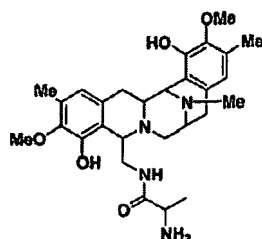
LRFABMS using m-NBA as matrix m/z (rel intensity) 509 $[M-H_2O+H]^+$ (5), 460 (2.7), 391 (3).

1H NMR (CD_3OD , 500 MHz): 6.70 (s, H-15), 6.52 (s, H-5), 4.72 (bs, H-11), 4.66 (d, J = 2.0 Hz, H-21), 4.62 (dd, J = 8.4, 3.7 Hz, H-1), 3.98 (bd, J = 7.6 Hz, H-13), 3.74 (s, 7-OMe), 3.71 (s, 17-OMe), 3.63 (m, overlapped signal, H-25), 3.62 (m, overlapped signal, H-3), 3.30 (m, H-22a), 3.29 (m, H-14a), 3.18 (d, J = 18.6 Hz, H-14b), 2.90 (m, H-4a), 2.88 (m, H-22b), 2.76 (s, 12-NMe), 2.30 (s, 16-Me), 2.22 (m, H-4b), 1.16 (d, J = 7.4 Hz, H-26);

^{13}C NMR (CD_3OD , 125 MHz): 170.75 (s, C-24), 149.24 (s, C-18), 147.54 (s, C-8), 145.95 (s, C-7), 145.82 (s, C17), 133.93 (s, C-16), 132.31 (s, C-9), 131.30 (s, C-6), 128.95 (s, C-20), 121.93 (d, C-15), 121.76 (d, C-5), 121.44 (s, C-10), 112.45 (s, C-19), 92.87 (d, C-21), 60.86 (q, 7-OMe), 60.76 (q, 17-OMe), 59.39 (d, C-11), 57.96 (d, C-13), 55.51 (d, C-1), 54.29 (d, C-3), 50.08 (d, C-25), 45.55 (t, C-22), 40.43 (q, 12-NMe), 32.56 (t, C-4), 25.84 (t, C-14), 17.20 (q, C-26), 16.00 (q, 16-Me), 15.81 (q, 6-Me).

Please replace page 41 of the specification as follows:

COMPOUND P-22A



Strain:

The same as for P-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

Biological activities of safracin P-22A

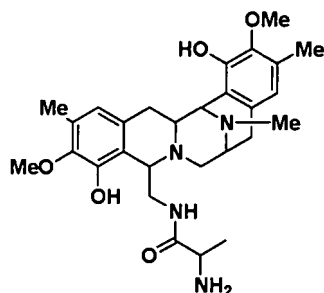
Antitumor activities

Primary Screen(s)	Cells Lines (Mol/L)															
	Panc-1		Ovary		Hep-2		Hep-3		Hep-4		Hep-5		Hep-6		Hep-7	
Safracin P-22A	625	> 1.95E-05	1.95E-05	7.24E-05	1.95E-05	1.27E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05
	125	> 1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05
	100	> 1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05	1.95E-05

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): NO ACTIVE

COMPOUND P-22A

*Strain:*

The same as for P-22B

Fermentation conditions:

The same as for P-22B

Isolation:

The same as for P-22B

Biological activities of safracin P-22A

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-22A	GI50	>1.96E-05	4.19E-06	7.74E-06	1.30E-05	1.27E-05	5.93E-06	>1.96E-05	3.15E-06	>1.96E-05	1.26E-05	>1.96E-05	>1.96E-05	8.75E-06	7.66E-06
	TGI	>1.96E-05	9.26E-06	1.96E-05	>1.96E-05	>1.96E-05	1.33E-05	>1.96E-05	7.93E-06	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.96E-05
	LC50	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05	>1.96E-05

Antimicrobial activity: On solid medium*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVE

Please replace page 43 of the specification as follows:

Biological activities of safracin P-19B

Antitumor activities

Primary Screening	Cells Lines (Mol/L)															
	Pilot		Dry		JES		MDA-MB-231		MDA-MB-231		MDA-MB-231		MDA-MB-231		MDA-MB-231	
	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX	TOX
safracin P-19B	650	1.8E-05	1.8E-05	5.8E-05	8.8E-05	7.8E-05	7.8E-05		1.8E-05	2.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05
23-04702	750	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05		1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05
23-04702	1250	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05		1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05	1.8E-05

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): NO ACTIVE

Spectroscopic data:

HRFABMS m/z 495.260410 $[M-H_2O+H]^+$ (calcd for $C_{27}H_{35}N_4O_5$ 495.260746 Δ 0.3 mmu); LRFABMS using m-NBA as matrix m/z (rel intensity) 495 $[M-H_2O+H]^+$ (13), 460 (3), 391 (2); 1H NMR (CD_3OD , 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, $J = 7.1$ Hz, H-26);

Biological activities of safracin P-19B

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin P-19B	GI50	1.70E-05	3.90E-06	5.42E-06	8.74E-06	7.08E-06	7.90E-06	>1.95E-05	2.38E-06	1.81E-05	1.55E-05	>1.95E-05	1.44E-05	6.73E-06	4.80E-06
	TGI	>1.95E-05	8.06E-06	1.48E-05	>1.95E-05	1.92E-05	>1.95E-05	>1.95E-05	5.77E-06	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.61E-05	1.00E-05
	LC50	>1.95E-05	1.67E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.40E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	>1.95E-05	1.95E-05

Antimicrobial activity: On solid medium*Bacillus subtilis*. 10µg/disk (6mm diameter): NO ACTIVESpectroscopic data:HRFABMS m/z 495.260410 $[M-H_2O+H]^+$ (calcd for $C_{27}H_{35}N_4O_5$ 495.260746 Δ 0.3 mmu);LRFABMS using m-NBA as matrix m/z (rel intensity) 495 $[M-H_2O+H]^+$ (13), 460 (3), 391 (2); 1H NMR (CD_3OD , 500 MHz): 6.67 (s, H-15), 6.5 (s, H-5), 3.73 (s, 7-OMe), 3.71 (s, 17-OMe), 2.29 (s, 16-Me), 2.24 (s, 6-Me), 1.13 (d, $J = 7.1$ Hz, H-26);

Please replace page 46 of the specification as follows:

Cells Lines (Mol/L)																
Primary Screening	Prostate		Ovary		Breast		Endothelial		Hep2		Pancreatic		Colon		Cervix	
	PC-4	PC-3	SK-OV-3	OV-10	MDA-MB-231	BT-20	ECV-109	ECV-109	MDA-MB-231	MDA-MB-231	PA-103	PA-103	HCT-116	HCT-116	HEP-2	HEP-2
PM - Fermento de la Cella 123	680	5.22E-03	1.54E-03	2.58E-03	1.33E-03	4.71E-03	2.51E-03		8.04E-03	8.04E-03	1.77E-03	4.33E-03	8.93E-03	4.33E-03	2.73E-03	2.73E-03
TSI	751	3.83E-03	4.43E-03	8.02E-03	3.34E-03	7.82E-03	8.21E-03		1.87E-03	1.87E-03	1.40E-03	1.79E-03	1.62E-03	8.82E-03	5.52E-03	5.24E-03
13-AC302	1620	1.53E-03	3.71E-03	1.32E-03	3.42E-03	1.32E-03	1.32E-03		1.81E-03	2.73E-03	> 1.50E-03	> 1.21E-03	> 1.21E-03	1.64E-03	1.83E-03	1.21E-03

Secondary Evaluation (Mol/L)											
Secondary Screening	Macromolecular Synthesis					Apoptosis		DNA Binding		Cytotoxicity	
	Protein	DNA	RNA	Apoptosis	Apoptosis	DNA Binding	DNA Binding	Cytotoxicity	Cytotoxicity		
PM - Fermento de la Cella 123 13-AC302	750	1.83E-03	1.82E-03	1.83E-03	2.83E-03	6.03E-03					

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS: m/z 509 $[M-H_2O+H]^+$; 1H NMR ($CDCl_3$, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safranin D	GI50	5.22E-06	1.54E-06	2.68E-06	1.33E-06	4.71E-06	3.51E-06	6.04E-06	6.04E-07	4.77E-06	4.33E-06	6.99E-06	4.75E-06	3.76E-06	2.28E-06
	TGI	9.99E-06	4.12E-06	6.02E-06	3.34E-06	7.82E-06	6.21E-06	1.07E-05	1.16E-06	1.10E-05	1.79E-05	1.82E-05	8.85E-06	6.68E-06	5.24E-06
	LC50	1.90E-05	9.78E-06	1.35E-05	9.15E-06	1.30E-05	1.10E-05	1.88E-05	3.78E-06	>1.90E-05	>1.90E-05	>1.90E-05	1.65E-05	1.19E-05	1.21E-05

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin D	IC50	1.90E-05	1.52E-05	3.80E-06	2.85E-06	6.65E-06

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): Inhibition zone: 15 mm diameter

Spectroscopic data

ESMS: m/z 509 $[M-H_2O+H]^+$; 1H NMR ($CDCl_3$, 300 MHz): 6.50 (s, C-15), 4.02 (s, OMe), 3.73 (s, OMe), 2.22 (s, Me), 1.85 (s, Me), 0.80 (d, $J = 7.2$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 186.51, 181.15, 175.83, 156.59, 145.09, 142.59, 140.78, 137.84, 131.20, 129.01, 126.88, 121.57 (2 x C), 82.59, 60.92, 60.69, 53.12, 21.40, 50.68, 50.22, 48.68, 40.57, 29.60, 25.01, 21.46, 15.64, 8.44.

Please replace page 48 of the specification as follows:

Cells Lines (Mo/L)																
Primary Screening	M. PROBAB.		Duty		Bioss		Bioss		ASL		POTASS		GEM		Cant	
	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.
PU - Fernando de la Cueva220	820	0.115-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05
	781	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05
13-400-02	1120	> 1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05	1.015-05

Secondary Evaluation (Mo/L)																
Secondary Screening	Metabolite Synthesis				Apoptosis		DNA Binding		Antitumor		Anticancer		Anticancer		Anticancer	
	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.	PROB.
PU - Fernando de la Cueva220 13-400-02 820																

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: m/z 511 $[M+H]^+$; 1H NMR ($CDCl_3$, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, $J = 6.6$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin E	GI50	8.34E-06	3.86E-06	4.50E-06	4.54E-06	5.05E-06	3.94E-06	1.96E-05	4.25E-06	6.05E-06	7.89E-06	7.15E-06	5.07E-06	4.15E-06	4.03E-06
	TGI	1.96E-05	7.70E-06	8.85E-06	8.25E-06	9.24E-06	6.93E-06	>1.96E-05	8.21E-06	1.47E-05	1.96E-05	>1.96E-05	9.44E-06	7.29E-06	7.25E-06
	LC50	>1.96E-05	1.54E-05	1.74E-05	1.49E-05	1.70E-05	1.22E-05	>1.96E-05	1.59E-05	>1.96E-05	>1.96E-05	>1.96E-05	1.75E-05	1.28E-05	1.30E-05

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin E	IC50			1.57E-05	>1.96E-05	

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6mm diameter): 9.5 mm inhibition zone

Spectroscopic data

ESMS: m/z 511 $[M+H]^+$; 1H NMR ($CDCl_3$, 300 MHz): 6.51 (s, C-15), 4.04 (s, OMe), 3.75 (s, OMe), 2.23 (s, Me), 1.89 (s, Me), 0.84 (d, $J = 6.6$ Hz); ^{13}C NMR ($CDCl_3$, 75 MHz): 186.32, 181.28, 175.83, 156.43, 145.27, 142.75, 141.05, 137.00, 132.63, 128.67, 126.64, 122.00, 120.69, 60.69, 60.21, 59.12, 58.04, 57.89, 50.12, 49.20, 46.72, 39.88, 32.22, 25.33, 21.29, 15.44, 8.23.

Please replace page 52 of the specification as follows:

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin B (OEt)

Antitumor activities

Primary Screening	Cells Lines (Mol/L)													
	Prostate		Ovary		Breast		Neuroblastoma		Colon		Leukemia		Cervix	
	DU145	PC9	SKOV-3	ESOV	MDA-MB-231	T-47D	SK-N-SH	SK-N-BE	HT-29	HT-20	HL-60	THP-1	MDA-MB-435	HEP-2
Safracin B OEt	0.05	4.91E-07	4.84E-08	4.08E-08	3.83E-07	4.82E-08	1.58E-07	5.21E-07	1.50E-08	6.43E-07	2.41E-07	4.43E-07	2.09E-08	3.22E-08
23-0242	10	1.91E-08	>1.73E-08	1.97E-08	1.83E-08	1.15E-07	4.43E-07	1.16E-08	1.09E-07	2.06E-08	1.35E-08	1.05E-08	3.88E-08	3.45E-07
23-0242	100	1.00E-04	3.29E-07	4.27E-08	3.37E-08	1.02E-08	1.13E-08	1.55E-08	2.53E-08	1.26E-08	>1.73E-08	>1.73E-08	1.26E-08	3.78E-07

Secondary Evaluation (Mol/L)													
Secondary Screening	Mammalian Tissues				Anticancer		Antibacterial		Antifungal		Antiparasitic		Other
	Prostate	Ovary	Breast	Neuroblastoma	Colon	Leukemia	Cervix	Anticancer	Antibacterial	Antifungal	Antiparasitic	Other	
10-0242	100	>1.73E-08	1.26E-08	1.26E-07	6.25E-08	1.73E-08	-	-	-	-	-	-	-

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

Spectroscopic data:

ESMS: m/z 551 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

cells. The clarified broth (765 ml) was adjusted to pH 9.0 by NaOH 10%. Then, the alkali-clarified broth was extracted with 1:1 (v/v) EtOAc (x2). The organic phase was evaporated under high vacuum and a greasy-dark extract was obtained (302 mg).

This extract was washed by an hexane trituration for removing impurities and the solids were purified by a chromatography column using Silica normal-phase and a mixture of Ethyl Acetate: Methanol (from 12:1 to 1:1). The fractions were analyzed under UV on TLC (Silica 60, mobile phase EtOAc:MeOH 5:4. Rf 0.3 (Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin B (OEt)

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin B (OEt)	GI50	4.01E-07	4.84E-08	4.06E-08	6.82E-07	4.82E-08	1.69E-07	5.01E-07	3.97E-08	6.49E-07	2.44E-07	4.43E-07	2.09E-06	8.92E-08	7.70E-08
	TGI	1.01E-06	>1.76E-05	9.97E-08	1.19E-06	1.16E-07	4.40E-07	1.16E-06	1.08E-07	2.06E-06	1.39E-06	1.09E-06	9.88E-06	3.15E-07	2.74E-07
	LC50	1.60E-05	8.28E-07	4.27E-06	6.37E-06	1.02E-06	1.13E-06	5.66E-06	3.69E-06	1.35E-05	>1.76E-05	>1.76E-05	>1.76E-05	1.35E-06	9.76E-07
Secondary Evaluation (Mol/L)															
Secondary Screening				Macromolecules Synthesis						Apoptosis			DNA Binding		
				PROTEIN		DNA		RNA		NUCLEOSOMES			GEL		
Safracin B (OEt)		IC50		>1.76E-05		1.76E-06		1.76E-07		5.28E-08			1.76E-05		

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 17,5 mm inhibition zone

Spectroscopic data:

ESMS: m/z 551 [M-H₂O+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.31 (s, 16-Me), 2.22 (s, 12-NMe), 1.88 (s, 6-Me), 1.43 (t, J = 6.9 Hz, Me-Etoxy), 1.35 (t, J = 6.9 Hz, Me-Etoxy), 0.81 (d, J = 7.2 Hz, H-26)

Please replace page 54 of the specification as follows:

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

Antitumor activities

Cells Lines (Mol/L)														
Primary Screening	Prostate		Ovary		Breast		Leukemia		HBL		Panc		Colon	
	PC14	PC9	OV10	OV20	MDA	MDA	HL60	HL60	HL60	HL60	HL60	HL60	HL60	HL60
Safracin A (OEt) [ED ₅₀]	GM	2.51E-08	2.20E-07	4.02E-07	2.91E-05	6.05E-07	7.93E-07	4.01E-05	2.11E-07	3.05E-05	1.97E-05	2.03E-05	6.22E-05	1.03E-05
23-3C42	GM	5.39E-08	7.12E-07	6.20E-07	4.10E-05	1.85E-05	1.90E-05	7.17E-05	6.85E-07	6.23E-05	4.41E-05	4.11E-05	8.81E-05	2.91E-05
	IC50	1.10E-05	1.45E-05	1.75E-05	1.20E-05	5.51E-05	4.37E-05	1.30E-05	1.51E-05	1.15E-05	3.88E-05	5.51E-05	1.81E-05	7.55E-05

Secondary Evaluation (Mol/L)									
Secondary Screening	Antimicrobial Susceptibility			Antitumor		DNA Binding		Cytotoxicity	
	EXTEN	NOVA	ISA	TOXENDES	NBE	ACTH	DELI	FLASK	FLASK
10-0140	ED			6.31E-05	1.81E-05				

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, J = 6.9 Hz, Me-Etoxy), 1.34 (t, J = 6.9 Hz, Me-Etoxy), 0.8 (d, J = 6.9 Hz, H-26)

EtOAc:MeOH 5:4. Rf 0.3 Safracin B-OEt and 0.15 Safracin A-OEt). From this, safracins B OEt (25 mg) and safracin A OEt (20 mg) were obtained.

Biological activities of safracin A (OEt):

Antitumor activities

Cells Lines (Mol/L)															
Primary Screening		Prostate		Ovary		Breast	Melanoma	NSCL	Leukemia	Pancreas	Colon			Cervix	
		DU-145	LN-caP	IGROV	IGROV-ET	SK-BR3	SK-MEL-28	A549	K-562	PANC1	HT29	LOVO	LOVO-DOX	HELA	HELA-APL
Safracin A (OEt)	GI50	2.64E-06	3.78E-07	4.92E-07	2.01E-06	5.55E-07	7.96E-07	4.00E-06	3.11E-07	3.06E-06	1.97E-06	2.03E-06	5.72E-06	1.02E-06	7.64E-07
	TGI	5.39E-06	7.42E-07	9.28E-07	5.10E-06	1.16E-06	1.90E-06	7.17E-06	6.86E-07	5.83E-06	4.41E-06	4.41E-06	9.84E-06	2.91E-06	2.32E-06
	LC50	1.10E-05	1.45E-06	1.76E-06	1.30E-05	5.57E-06	5.77E-06	1.28E-05	1.51E-06	1.11E-05	9.88E-06	9.88E-06	1.69E-05	7.85E-06	6.69E-06

Secondary Evaluation (Mol/L)						
Secondary Screening		Macromolecules Synthesis			Apoptosis	DNA Binding
		PROTEIN	DNA	RNA	NUCLEOSOMES	GEL
Safracin A (OEt)	IC50			6.33E-06	1.81E-06	

Antimicrobial activity: On solid medium

Bacillus subtilis. 10µg/disk (6 mm diameter): 10 mm inhibition zone

Spectroscopic data:

ESMS: m/z 553 [M+H]⁺; ¹H NMR (CDCl₃, 300 MHz): 6.48 (s, H-15), 2.33 (s, 16-Me), 2.21 (s, 12-NMe), 1.88 (s, 6-Me), 1.42 (t, *J* = 6.9 Hz, Me-Etoxy), 1.34 (t, *J* = 6.9 Hz, Me-Etoxy), 0.8 (d, *J* = 6.9 Hz, H-26)